

Detection of Cytotoxic Anti-LEDGF Autoantibodies in Atopic Dermatitis

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In the last two decades, atopic dermatitis (AD) has been of increasing clinical significance in Japan. Eight–20% of patients with AD developed progressive cataracts (cataract-AD) and lens epithelial cells (LECs) were severely damaged. Lens epithelium-derived growth factor (LEDGF) is a newly isolated survival factor. In the presence of LEDGF, LECs survive well and in the absence of LEDGF, they become highly susceptible to stress. We investigated (1) whether auto-antibody (auto-Ab) to LEDGF is present in sera of AD patients and (2) whether depletion of LEDGF by the auto-Ab kills LECs. In sera from 26 patients with AD using ELISA, we found significantly higher levels of auto-Ab to LEDGF than that in a normal control group. Affinity purified auto-Ab to LEDGF from these sera killed LECs without complement activation. Levels of histamine in the AD group were significantly higher and levels of prostaglandin E₂ were significantly lower than in the normal group. However, statistically there are no differences between sera from AD and cataract-AD in levels of Ab to LEDGF, histamine, prostaglandin E₂ (PGE₂), immunoglobulin E (IgE) and eosinophiles. We speculate that cataract-AD may be induced, in part, by a combination of high levels of serum histamine and eye rubbing which could break the blood-aqueous barrier to allow the entry of Ab to LEDGF into the privileged compartment, thus, reducing LEDGF levels, resulting in damage to LECs, and cataract formation.

Keywords: Atopic dermatitis; Auto-Ab to LEDGF; Cataract; Histamine; Lens epithelium-derived growth factor (LEDGF) and cell death

Abbreviations: AD, atopic dermatitis; Cataract-AD, AD patients having cataract; DMEM, Dulbecco's modified eagle medium; LECs, lens epithelial cells; LEDGF, lens epithelium-derived growth factor

INTRODUCTION

Atopic dermatitis (AD) is a common, chronic, intermittent, inflammatory skin disease of children, adolescents and young adults.^[1,2] A skin lesion develops primarily in the epidermis and chronic scratching and rubbing lead to lichenification, typically in the flexures of the elbows and knees. Bacterial and viral infections in skin are common in AD. AD is considered to be a multifactorial disease and both hereditary and environmental factors are believed to contribute. Most patients have a family history of one or more allergic conditions.^[3,4] The hereditary nature of AD has been recognized for many years and it has been recently confirmed that the gene encoding immunoglobulin

E (IgE) responsiveness in AD is located on chromosome 11q.^[5,6] Studies that favor a dominant, recessive or polygenic inheritance have all been presented, but the multifactorial nature of the disease condition cannot be denied.^[1,2] The environmental factors are considered to be allergens.^[3,4,7] Abnormal humoral immunity underlies this disease and IgE is usually prevalent at the site of local inflammation.^[1,2] The exact relationship between AD and IgE is not clear. A number of clinical and immunological findings suggest that cell-mediated immunity is depressed in AD.^[8] In contrast, Leung *et al.*^[9] reported that cell-mediated cytotoxicity plays a role against skin fibroblasts in AD patients. AD is frequently accompanied by asthma, allergic rhinitis and

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ocular symptoms including cataract, keratoconus and keratoconjunctivitis and retinal detachment.^[3,4]

Incidence of cataract in AD patients ranges from 8 to 24%.^[10–14] Atopic cataracts are bilateral and progress rapidly in many cases. Fagerholm *et al.*^[13] and Sasaki and Kojima^[14] reported significant degeneration and decrease in density of lens epithelial cells (LECs) in AD patients with cataract. Recently, Mihara *et al.*^[15] reported that apoptotic cell death of LECs is significantly higher in cataract-AD than senile cataract.

Previously, we have isolated a lens epithelium-derived growth factor (LEDGF), which stimulated cell adhesion, growth and antiapoptosis in a wide range of cell types.^[16] We observed that in the presence of LEDGF, cells survived well but in the absence of or in the presence of very low levels of LEDGF, many cell types including, keratinocytes, fibroblasts, LECs, cos7 cells, neuroretinal cells and corneal cells die.^[17–19] Interestingly, intrinsic and extrinsic supplies of LEDGF activate expression of stress-related proteins including heat shock proteins (Hsps). Higher levels of Hsps negatively regulate activation of caspases-3 and result in suppression of apoptosis.^[20] In contrast, in the absence of LEDGF, expression of Hsps is suppressed and activation of caspases-3 is stimulated and resulting in apoptosis.^[21–25] In addition, we found a depletion of LEDGF with Abs to LEDGF induced cell death.^[17–19] LEDGF is a heparin binding protein and heparin-LEDGF complexes are highly stable but heparin-free LEDGF is highly susceptible to proteolysis.^[26]

We investigated whether the sera from patients with cataract and AD (cataract-AD) have cytotoxic Abs to LEDGF. Our results showed that auto-antibody (auto-Ab) to LEDGF was present in sera from AD and cataract-AD and it was cytotoxic to LECs. *However, there is no significant difference in levels or cytotoxic capacity of antibodies between AD and cataract-AD patients.* Also, high levels of histamine were detected in the sera from AD and cataract-AD patients suggesting that permeability of the ocular-blood barrier might be increased. *Again, there is no significant difference in histamine levels is seen between AD and cataract-AD groups.* We will further, discuss, the possible mechanisms underlying cataract formation in AD patients.

MATERIALS AND METHODS

Cases

We recruited 26 patients with AD who were diagnosed by dermatologists following well-defined criteria^[27] and randomly picked 12 cataract and 14 non-cataract patients for present study. Informed consent was obtained from all subjects. All had refrained from taking corticosteroids. Healthy donors had no personal or family history of AD. Complete ophthalmological examinations were done by ophthalmologists, including slit lamp biomicroscopy and binocular ophthalmoscopy. The diagnosis of atopic

cataract was made by the presence of typical anterior/posterior sub capsular cataract.

Serum

Approximately 10 ml of peripheral blood was collected after obtaining informed consent. They were centrifuged at 3000g for 20 min. Serum was collected, aliquoted in 0.5 ml/tube and kept in -70°C until use.

LEC Culture and Cytotoxicity Assay

LECs with the capsule from 1.5-month-old mice were trypsinized for 30 min as described elsewhere.^[28] The cells were dissociated and cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco-BRL, Grand Island, NY) with 10% heat inactivated fetal calf serum (FCS; Gibco-BRL) for a cytotoxicity test. The LECs were cultured at 37°C in an atmosphere of 5% CO_2 . After 2–3 days culture, cells were harvested and 50,000 cells were plated in 96-well microtiter plates. Cell death with complement activation assay: the cell viability was analyzed with a trypan blue dye exclusion test after 3 h incubation in various amounts of human serum.^[29] We added guinea pig complement (Gibco-BRL) at the dilution of 1/20. Without complement, the cell viability was analyzed with a trypan blue dye exclusion test after 2–3 days incubation in various amount of Ab to LEDGF. All experiments were carried out in three microtiter wells and performed in triplicate. Some cytotoxic experiments were carried out with sera, which had been purified by affinity purification (see below).

ELISA

The Ab titration was carried out by the indirect enzyme-linked immunosorbent assay (ELISA).^[30] Purified GST-LEDGF was used as an antigen, and only GST was used as a control Ag. The titer, expressed in optical density (OD_{490}) units, was calculated as a multiple of the dilution in the linear portion of the standard plot.

Histamine and Prostaglandin E_2 (PGE_2) Levels in the Serum

The concentration of the total histamine and PGE_2 in serum was determined with ELISA kits provided by a commercial source (histamine kit from Immunotech, Westwood, ME and prostaglandin detection kit from Assay designs, Incorporation, Ann Arbor, MI) following the manufacturer's protocols. All other laboratory tests were done at each hospital.

Protein Blot Analysis

GST-LEDGF purified from prokaryotic expression system^[16,17,20] was dissolved in SDS-PAGE sample buffer, run on 10% SDS-PAGE, and blotted onto Immobilon™-P (Millipore Corporation, Bedford, MA).

The filters were preincubated with 7% (w/v) skim milk in PBS at 4°C overnight and then incubated for 2 h at room temperature with rabbit Abs to LEDGF (C-terminal peptide) as primary Abs at 1:1000 dilution in PBS containing 0.05% Tween 20 (PBS-T) and 1% (w/v) skim milk. The filters were washed three times with PBS-T and incubated with anti-rabbit IgG labeled with horseradish peroxidase (Santa Cruz Biochemistry, CA; 1:1000 dilution, 200 ng/ml) as a second Ab. The color was developed with 0.04% hydrogen peroxide and 0.05% DAB. To confirm specificity of the Abs to LEDGF, the Ab was neutralized with the same volume of purified GST-LEDGF (1 µg/µl) overnight at 4°C. Protein size markers were purchased from Bio-Rad Laboratories, Incorporation.

Affinity Purification of Human Auto-Abs to LEDGF

A 50 µg of LEDGF was transferred to a nitrocellulose filter. A 1.0 cm wide horizontal strip of membrane containing 10 µg LEDGF was incubated overnight with 2.5 ml of human serum diluted 1/250 with Tween 20 (1%) in 10 mM Tris-HCl, containing 0.15 M NaCl (pH 7.4) (TBS). Then the strips were washed three times with 0.5% Tween 20/TBS, then washed again briefly with water, and then the Abs were eluted twice at 37°C for 30 min with a solution of 50 mM glycine, 1 M NaCl (pH 2.3). The eluate was collected, neutralized and kept on ice. The eluate from four horizontal strips was dialyzed against TBS, then concentrated to about 4 ml on an Amicon PM-10 filter (Amicon Corporation, Lexington, MA), and further concentrated to about 50 µl on a micro concentrator Centricon 30 (Amicon Corporation).

Statistical Analysis

We applied statistical analysis to each parameter using "Basic Statistics I" of Medical Software Library (System Information Center Incorporation, Japan).

RESULTS

Abs to LEDGF in Serum from AD and Cataract-AD

Sera from 26 AD patients and from 12 non-AD individuals were collected. Among the 26 AD patients, 12 had cataract and 14 had no cataract when blood samples were drawn. The types of cataracts are indicated in Table I. Sera from AD patients killed an average of $23.1 \pm 4.1\%$ mouse LECs at dilution of 1/20 and from non-AD patients killed $4.8 \pm 1.1\%$, ($p < 0.005$, Student *t*-test). However, no difference was observed in serum cytotoxicity between AD ($21.4 \pm 4.6\%$) and cataract-AD ($24.8 \pm 3.7\%$) (Fig. 1). Each test was conducted using serial dilutions of serum (1/7, 1/20 and 1/60) and there was a linear dose response curve.

These results indicated that serum from AD patients had more Abs than that of normal people since it exhibited higher cytotoxicity.

Next, we explored the cytotoxicity of Abs, in which complement fixing is not involved. Mouse LECS were cultured in the presence of heat-inactivated sera (56°C for 30 min) for 3 days, which exhibited 3–10% of cytotoxicity compared to complement fixed cytotoxicity. We speculated that cytotoxic Abs such as Abs to LEDGF might be involved in the cytotoxicity of sera from AD-patients.

We next quantified auto Ab to LEDGF in sera from AD patients with the indirect ELISA. Abs to LEDGF in the cataract-AD (all of 8 patients) and AD without cataract (7 out of 13) had high Ab levels ($> 30 \text{ OD}_{492}$) (Table I). A statistically significant difference of Ab levels, however, was not found between sera from AD and cataract-AD ($p < 0.5607$, Student *t*-test).

To learn the prevalence of auto Ab to LEDGF in the general population, we obtained blood samples from a Blood Bank (Chicago Blood Bank) and quantified auto Ab to LEDGF. We analyzed 650 sera from the Blood Bank (presumably normal population) and majority of sera (95%) contained $< 30 \text{ OD}_{492}$, which was below base line. Only 5.4% had $> 30 \text{ OD}_{492}$ (Fig. 2). We found only one serum out of the 650 contained $> 115 \text{ OD}_{492}$ units which is a significantly high titer. *No significant difference was observed among age groups in these 650 blood bank samples, but the average levels of Ab from female to LEDGF was 5.0 OD₄₉₂ units higher than that of male.* However, a significant difference was observed between sera from AD and general population ($p < 0.002$, Student *t*-test).

We further tested the reliability of ELISA titer using a protein blot analysis. Purified GST-LEDGF was separated on SDS-PAGE and blotted onto an Immobilon™-P membrane; each strip of the membrane was then immunostained with serum, which had the highest anti-LEDGF titer ($\text{OD}_{490} \text{ unit} > 115$) in from the blood bank samples. We found a single GST-LEDGF (90 kDa) band stained strongly indicating that human serum does indeed have an auto-Ab to LEDGF. Similarly, blotted filter strips were immunostained with human sera from AD patients. LEDGF was positively immunostained in four sera out of 26 AD patients (see Table I: Serum No. 201, 221, 222 and 224) indicating that these sera have auto Abs to LEDGF detectable by protein blot analysis (Fig. 3). Four positive sera exhibited generally high ELISA titers but some sera which exhibited high titers by ELISA did not show immunostaining on the protein blot analysis suggesting that ELISA titer may include some nonspecificity (see Table I).

Auto-Ab to LEDGF Found in the Human Sera Killed LECs

An affinity-purified auto-Ab to LEDGF from the Blood Bank serum (115 OD_{492} units) killed more than 60% of mouse LECs after a two-day exposure. Similarly, three

TABLE I Summary of age, gender, cataract condition of patients and levels of serum components

Serum	Age sex	Cataract type	Cell death (%)	Histamine (nM)	PGE ₂ (pg/ml)	IgE (mg/dl)	Eosinophile (%)	Ab to LEDGF (OD)
<i>AD with cataract group</i>								
215	15F	Mature	50 + 8	119.0	260	2900	4.2	121.0
109	26M	Mature	36 + 3	45.0	204	980	11.0	N/C
403	16F	ASC + PSC	34 + 9	7.0	20	11,800	3.0	55.9
301	17M	ASC + PSC	34 + 2	27.0	700	N/C	N/C	N/C
224	30M	ASC + PSC	33 + 4	5.6	115	N/C	N/C	162.0*
208	16F	PSC	23 + 4	66.0	390	7200	9.3	103.4
210	19F	ASC + PSC	22 + 6	68.0	860	4900	14.2	56.2
216	16M	ASC + PSC	18 + 6	34.0	32	5923	8.6	N/C
205	25F	ASC + PSC	17 + 3	15.0	140	N/C	N/C	N/C
221	10M	PSC	13 + 0	28.0	220	150	7.5	158.6*
202	21M	ASC	12 + 4	35.0	0	15,000	27.4	34.4
220	8M	PSC	5 + 2	22.0	200	N/C	N/C	37.4
<i>AD group</i>								
218	19F		64 + 6	17.0	170	N/C	N/C	22.4
201	14F		35 + 4	24.0	40	390	7.9	102.6*
206	3F		33 + 9	50.0	540	320	22.5	39.6
219	17F		31 + 3	26.0	250	N/C	N/C	1.6
211	9F		29 + 5	37.0	2300	1000	13.5	36.6
223	18F		23 + 1	71.0	82	N/C	N/C	186.8
209	14F		22 + 5	16.0	1200	2400	11.7	N/C
225	6M		19 + 6	30.0	74	860	6.7	90.9
204	3F		12 + 6	18.0	76	7400	12.1	16.7
217	11M		12 + 0	41.0	120	N/C	N/C	61.8
213	14M		8 + 5	33.0	30	2600	15.8	9.1
203	8M		6 + 9	105.0	25	N/C	N/C	0.0
222	8M		3 + 5	25.0	110	4600	12.0	149.4*
212	3M		0 + 0	33.0	98	N/C	N/C	22.3
<i>Control group</i>								
C1	28M		3 + 3	2.9	280			
C2	17M		2 + 2	6.6	840			
C3	22F		6 + 0	19.2	840			
C4	29F		3 + 5	8.9	1200			
C5	28M		8 + 4	3.4	74			
C6	24F		2 + 2	5.8	250			
C7	20F		0 + 0	5.9	2500			
C8	14F		15 + 2	5.8	3300			
C9	22M		2 + 2	3.8	1750			
C10	16M		5 + 3	4.4	2000			
C11	31F		9 + 6	3.3	1500			
C12	33M		3 + 9	8.9	2000			

ASC, anterior sub-capsular cataract; PSC, posterior sub-capsular cataract; F, female; M, male; and NC, not counted.

*Ab to LEDGF was detected by protein blot analysis (Fig. 3).

affinity purified sera from AD patients with cataract killed $44.2 \pm 2.4\%$ (Serum No. 201), $32.4 \pm 5.7\%$ (Serum No. 222) and $32.9 \pm 5.2\%$ (Serum No. 221) of LECs after a two-day exposure. The amount of purified Ab to LEDGF in these sera was unknown, since it was too low to be detected by the Bradford method.^[31] In a control experiment, affinity-purified Abs from low-titer sera (10 sera were pooled) killed less than 1% of LECs. These results indicated that most human sera had minute level of Ab to LEDGF, but that those with high titers exhibited cytotoxicity to mouse LECs in culture.

Histamine Levels

Histamine levels in the sera were 38.5 ± 8.1 nM in the cataract-AD group, 37.6 ± 6.5 nM in the non-cataract AD group, and 6.5 ± 1.2 nM in the control group (Fig. 4). A significant difference was observed between sera from

the AD and control groups ($p < 0.0008$, Student *t*-test), however, no difference was observed between the AD and cataract-AD groups ($p = 0.9293$).

PGE₂ Levels

PGE₂ levels in the sera were 261.8 ± 72.6 pg/ml in the AD-cataract group, 365.4 ± 170.7 pg/ml in the AD group, and 1377.8 ± 266.5 pg/ml in the control group (Fig. 5). A significant difference was observed between sera from the AD and control groups ($p < 0.001$, Student *t*-test), however, no difference was observed between the AD and cataract-AD groups ($p = 0.6060$).

Eosinophile Counts and Immunoglobulin Levels

Eosinophile counts were $13.1 \pm 3.6\%$ in the AD-cataract group and $12.8 \pm 1.7\%$ in the AD group (normal < 6.8). Serum IgE levels were 5317 ± 1851 mg/dl in

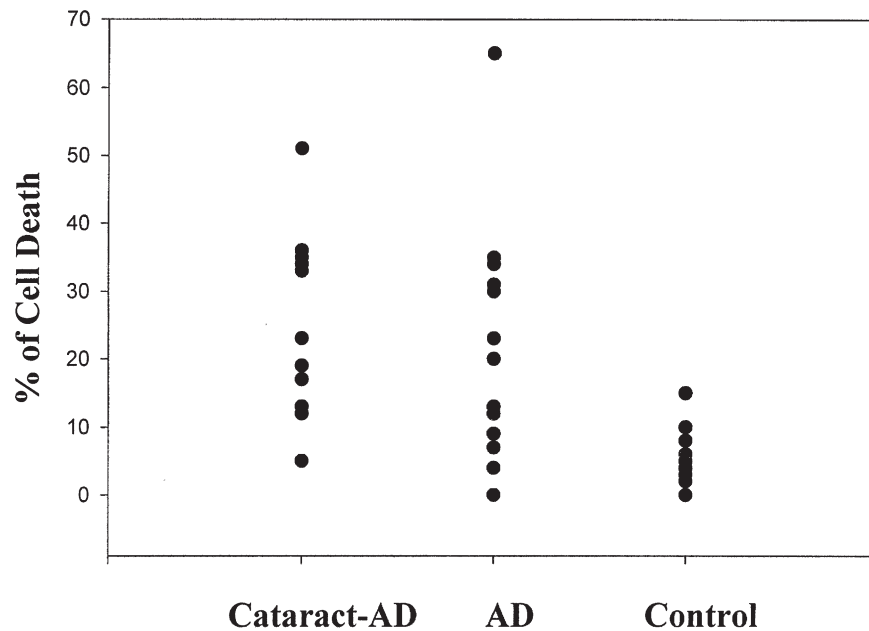


FIGURE 1 Sera from patients with AD or cataract AD killed LECs in culture. Mouse LECs were cultured in the presence of sera from patients or normal individuals with the addition of guinea pig complement. The percent of cell death was quantified by counting trypan blue positive and negative cells. Each dot indicates percent of LEC death with each serum sample.

the AD-cataract group and 2446 ± 871 mg/dl in the AD group (normal < 500). Serum immunoglobulin G levels were 1419 ± 267 mg/dl in the AD-cataract group and 1009 ± 110 mg/dl in the AD group (normal < 1800). Serum immunoglobulin M levels were 159 ± 30 mg/dl in

the AD-cataract group and 157 ± 37 mg/dl in AD group (normal < 200). Thus, eosinophile counts and IgE levels are higher than normal, but we did not demonstrate only significant differences between the AD-cataract and AD groups in any of tests.

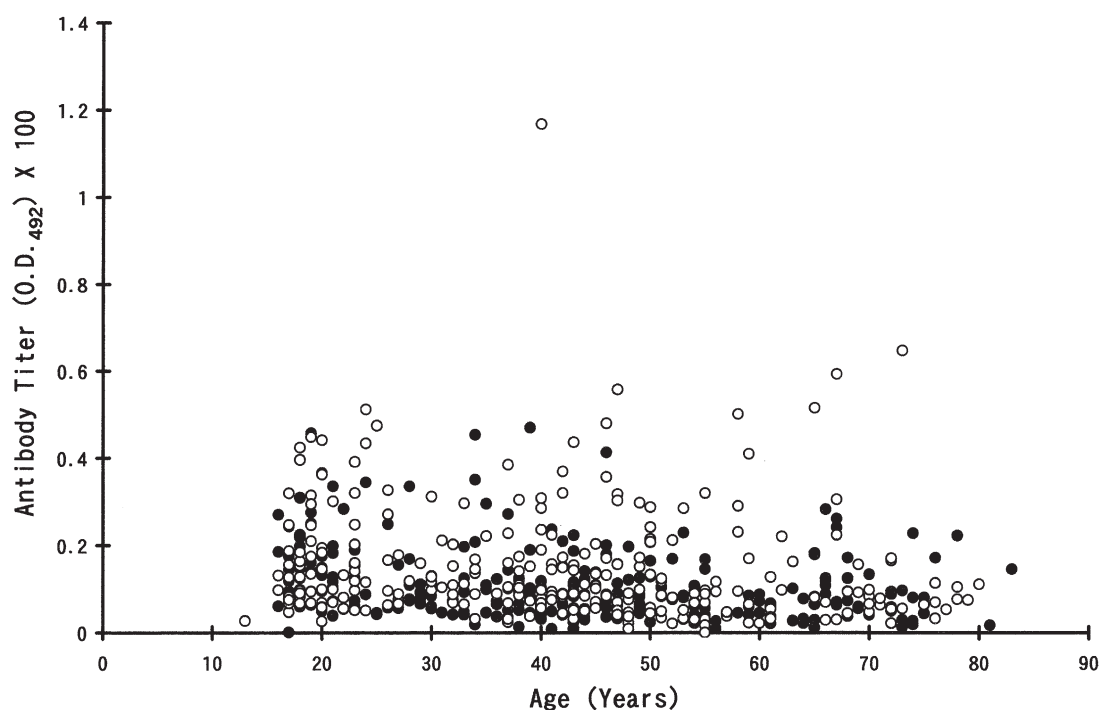


FIGURE 2 Auto-Abs to LEDGF in human serum quantified by ELISA analysis. Human sera, 650 samples from Chicago Blood Bank, were assayed with purified GST-LEDGF. Positive anti-GST titers were subtracted from anti-GST-LEDGF titers. Each experiment was done in triplicate, and each dot represents the mean of the three values for one serum. Each white and black circle indicates female and male, respectively. The abscissa indicates ages of individual from whom control sera were collected.

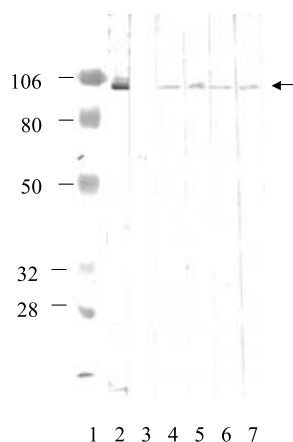


FIGURE 3 Protein blot analysis of GST-LEDGF with Abs from each patient with AD. Purified GST-LEDGF was used as antigen and affinity purified sera from each patient was used as probe. Lane 1—size markers from top 108, 80, 51, 34, 27, and 16 kDa, lane 2—highly purified GST-LEDGF (GST = 30 kDa; LEDG 60 kDa) stained a rabbit anti-LEDGF Ab (1/2000 dilution), lane 3—glutathione-S-transferase from *E. coli* transfected with vector plasmid, lane 4—sera from patient No. 224, lane 5—sera from patient No. 201, lane 6—sera from patient No. 221 and lane 7—sera from patient No. 222. An arrow indicates GST-LEDGF. The secondary Abs to the human or rabbit IgG was diluted 1/5000.

Cytotoxicity, serotonin, and IgM were higher in females ($p = 0.0038$, $p = 0.0431$, $p = 0.0136$, student *t*-test). Cytotoxicity was positively correlated with age ($r = 0.4309$, $n = 26$, $p < 0.05$), Pearson's product-moment correlation coefficient and IgM ($r = 0.6666$, $n = 14$, $p < 0.001$). Ab to LEDGF was negatively correlated with eosinophile count ($r = -0.6278$, $n = 15$, $p < 0.05$) and histamine level had no correlation to the other factors we have tested.

Statistical Analysis

Auto-Ab to LEDGF in AD patients is apparently higher than that of control (AD vs. control, $p = 0.0019$), but we cannot find any correlation between cataract-AD vs. non-cataract-AD ($p = 0.5607$). Age appears to have a significant role in developing cataract among AD patients ($p < 0.0045$) (Fig. 6) and older AD patients developed more cataract than younger AD patients suggesting that cataract development might take long time after an initial damage was done. Alternatively, age factors are deeply involved in cataract genesis. In addition, atopic cataract developed more in young female than in young males. The other factors tested such as PGE_2 ($p = 0.6060$), serotonin ($p = 0.1333$), eosinophile ($p = 0.7551$), IgE ($p = 0.1964$), IgG ($p = 0.0917$) and IgM ($p = 0.9762$), did not show significant differences between the AD and control groups.

DISCUSSION

Since the LEDGF clone was isolated with serum from a patient with age-related cataract, we expected to find auto-Abs to LEDGF in human serum.^[17] Among 650 human sera from the Blood Bank, about 95% of sera contained undetectable levels of auto-Ab to LEDGF, but 5% of sera contained very low levels of auto-Ab to LEDGF. We do not know the personal background of the serum from the Chicago Blood Bank, which had a relatively high level of Ab to LEDGF (more than 115 OD₄₉₀ units). In addition, Abs to LEDGF are found at higher levels in AD and AD-cataract patients. Although the exact amount of the affinity purified auto-Abs to LEDGF is unknown, it was sufficient to be toxic to mouse LECs and killed 32–44% of the cells

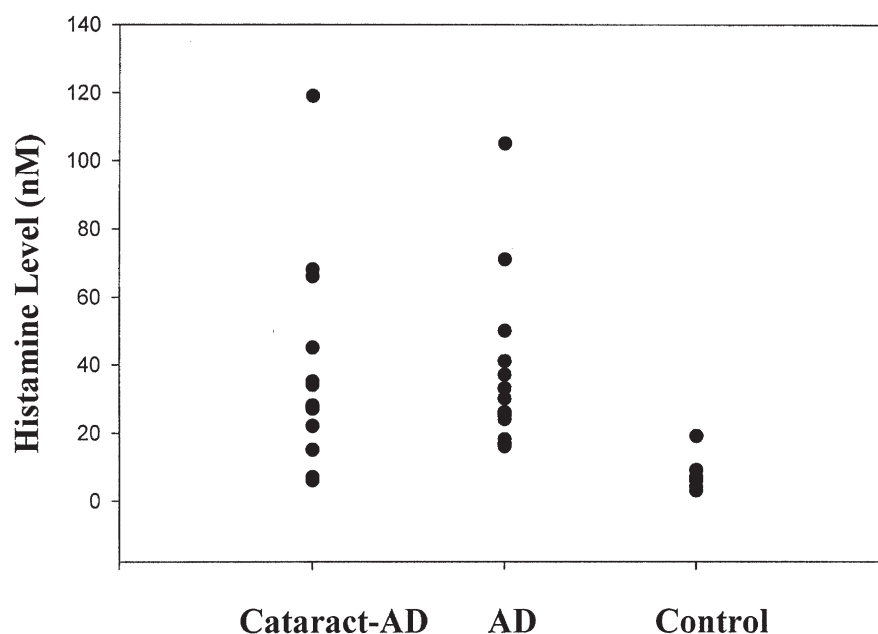


FIGURE 4 A significant difference was found in histamine levels between patients with AD and normal controls.

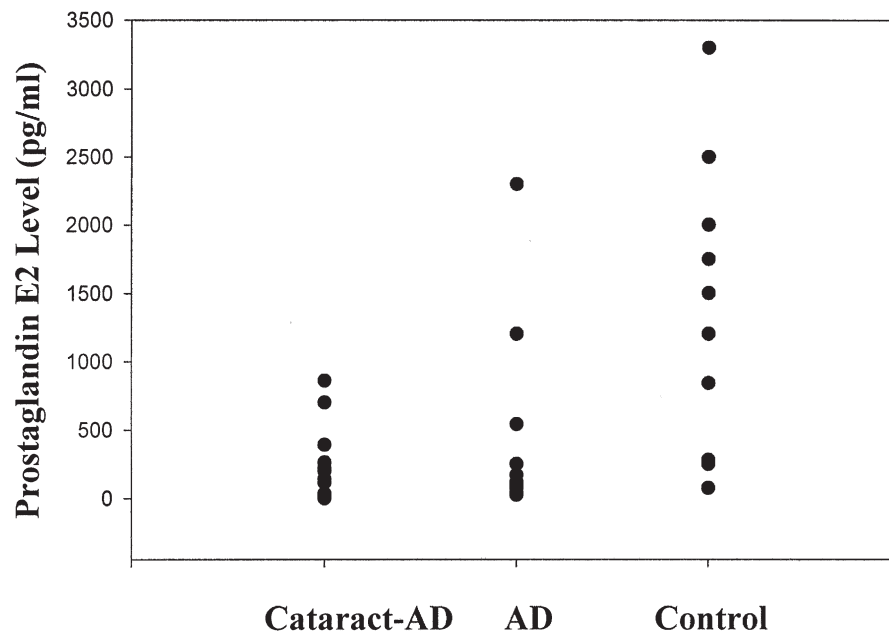


FIGURE 5 A significant difference was found in PGE2 levels between patients with AD and normal controls. The average concentration of PGE2 in the control 1377 ± 985 pg/ml, in no cataract-AD patients was 365 ± 639 and in cataract-AD patients, it was 261 ± 268 .

after a two-day exposure. In a control experiment, an affinity-purified Ab from an equal volume of low-titer sera killed less than 1% of LECs. These results indicated that most human sera have no detectable levels of auto-Ab to LEDGF, but that those with high titers are potentially damaging to LECs. Death of LECs in the cataract-AD was well documented by Mihara *et al.*^[15] Sasaki *et al.*^[32] and Majima *et al.*^[33] in the last few years. LEDGF is a survival factor for a wide range of cell types including LECs. Furthermore, depletion of LEDGF by rabbit Abs to LEDGF killed LECs, keratinocytes, and fibroblast in culture^[17–20] suggesting that auto Ab to LEDGF may play

some role in LEC death. Another investigator also reported that Ab to LEDGF was detected in the serum from uveitis (Voght-Koyanagi-Harada) patients (personal communication with Dr Yasuharu Nishimura, Kumamoto University, Japan).

We do not know how cytotoxic auto Ab to LEDGF was raised in the patients. Many exogenous Ags such as food, insect venoms or pollens are identified as allergens of hypersensitivity in AD.^[1–4] Antibodies can be raised by hypersensitivity reaction to the above allergens through inflammatory lesions in skin or infectious microbes having cross-reactive Ags. Interestingly, serum cytotoxicity to

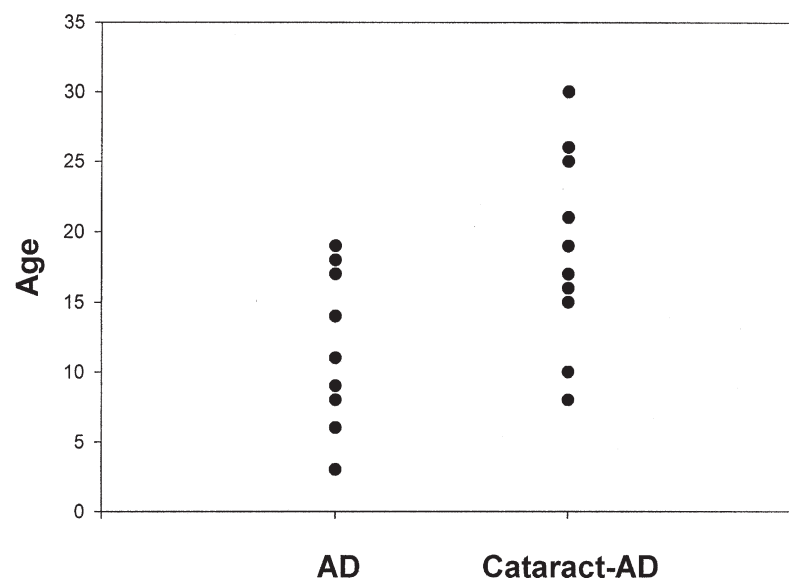


FIGURE 6 A statistically significant difference was found in age between non-cataract AD and cataract-AD patients.

LECs positively correlated with IgM, which may be produced after skin infection. We plan to investigate how auto Ab to LEDGF is developed in AD patients.

The anterior chambers are known to be privileged compartments and the lens is normally secluded from the circulation by the blood-ocular barrier.^[34,35] In AD patients, IgE and Ab complexes bind to mast cells and stimulate them to release chemotactic agents including histamine, prostaglandin, and bradykinin in local sites of inflammation.^[36] Under the influence of histamine,^[36] prostaglandins,^[37,38] and bradykinin,^[39] there is a breakdown in the barrier indicated by the entry of massive amounts of plasma proteins into the aqueous humor and a sharp rise of intraocular pressure.^[40] Worm *et al.* describes increased vascular permeability in AD using radio labeled IgG.^[41] An injection of bradykinin (an agonist) into laboratory animals increased vascular permeability of iris vessels.^[42,43] Nakano *et al.* studied levels of proteins in the aqueous humor of AD patients using a laser flare meter, and 23% (42/186) of AD patients exhibited elevated values.^[44] Although histamine does not increase the permeability of the blood-retinal barrier, it does increase the permeability of the iris capillaries, choriocapillaries, and ciliary vessels.^[45] *Since there is no significant differences in serum levels of histamine and Ab to LEDGF between AD and cataract-AD groups, we believe that rubbing due to pruritis in AD patients may break down further the blood aqueous barrier and allow auto-Abs into the anterior chamber.*^[46]

LEDGF is a heparin binding protein and it is stable in LEDGF-heparin complexes but heparin-free LEDGF is highly susceptible to proteolysis.^[26] The Ab to LEDGF raised in rabbit^[17] did not recognize heparin-GST-LEDGF complex but does bind to heparin-free GST-LEDGF. Since LEDGF is a secretory protein,^[16] secreted LEDGF in the cellular matrix might bind to the Ab instead of heparin.^[17] Our earlier work clearly indicated that a rabbit Ab to LEDGF killed human LECs in culture.^[17]

Our data indicated that PGE₂ levels in the sera from AD patients was much lower than that of controls and prostaglandin is known to open the tight junctions of ciliary epithelial cells.^[42] It has been assumed that the endogenous formation of PGE₂ is impaired in AD.^[47] However, PGE₂ concentrations in the plasma do not reflect the *in vivo* PGE₂ formation due to their short half-life (less than 1 min) and *in vivo* artifacts during blood sampling.^[48]

Eosinophils are known to release basic proteins (molecular size: 18–21 kDa), which are considered to be cytotoxic,^[49,50] and Yokoi^[51] reported the deposition of major basic proteins in LECs of atopic cataracts. However, in our studies, proteins smaller than 100 kDa in the present serum did not contain major cytotoxic factors (data not shown). Thus, basic proteins from eosinophils may not be major cytotoxic factors.

Cell death caused by complement fixing Abs is highly unlikely since no inflammation in the ocular chamber was detected in any of the AD-cataract patients (26 patients). In addition, ocular fluid contains complement inhibitor,^[52]

and no lymphocytes were drawn into ocular chamber.^[35,53]

Finally, cataract in juveniles and young adults are serious. AD in certain patients starts at very young age and lasts for several years, or even for life span. The duration of AD suggests that risk of cataract must be very high. Indeed older AD patients are more likely to have cataract than younger ones (see Fig. 6). The relationship between auto Ab to LEDGF and cataract-AD is under further investigation in our laboratory.

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